IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Hadlaczky et al.	Art Unit	: 1638
Serial No.	: 09/724,726	Examiner	: Helmer, G.L.
Filed	: November 28, 2000	Cust No.	: 20985
Conf. No.	: 7776		
Title	: *ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS FOR PREPARING ARTIFICIAL CHROMOSOMES*		

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Steven F. Fabijanski declare as follows:

1) I am familiar with the subject matter of the above-captioned application, which was filed on November 28, 2000, as well as the parent applications, including the earliest application U.S. Application Serial No. 08/629,822.

2) I have reviewed the Office Action, mailed March 30, 2006, in connection with the above-captioned application.

3) I received a Bachelor's degree in Biology from the University of Miami (Florida) in 1977. I received a Ph.D. degree in Cellular and Molecular Biology from the University of Southern California in 1981. I have held post-doctoral positions at the University of Ottawa in Ottawa, Ontario, Canada and the University of Southern California in Los Angeles, Califonia from 1982 to 1985. From 1986 to 1991, I held the position of Research Director at Paladin Hybrids, Inc.

4) I have over 20 years of experience in the area of plant molecular biology, plant gene expression, plant tissue and cell culture and development of techniques to produce genetically modified plants and plant artificial chromosomes. I have authored or co-authored over 20 publications and I am an inventor of over 16 US and foreign patents.

5) I am currently Director of Research and Development at Agrisoma Biosciences Inc., located in 8081 Lougheed Highway, Burnaby, B.C., Canada V5A 1W9. Chromos Molecular Systems, Inc., located at 8081 Lougheed Highway, Burnaby, B.C., Canada V5A 1W9, is an owner of Agrisoma Biosciences, Inc., to whom the subject matter of this application has been licensed.

6) I have held the position of Director since 2001. I am also President of the FAAR Biotechnology Group, Inc., located at Suite 323, 5929L Jeanne D'arc Boulevard, Orleans, Ontario, Canada K1C 7K2. I have held this position since 1992.

7) The above-captioned application describes the generation of satallite artificial chromosomes (designated SATACs in the application, and presently referred to as ACes), including plant SATACs. As described in the above-captioned application, SATACs, including plant SATACs, are generated following introduction of nucleic acid into the heterochromatic pericentric region of the chromosome, whether by targeted introduction or random introduction, which initiates amplification events leading to the generation of the *de novo* centromere, and ultimately of a SATAC. For example, as described in the above-captioned application at page 6, line 15 to page 7, line 23, SATACs are generated by introduction of heterologous nucleic acid encoding one or more selectable markers into cells, growing cells, and identifying from the resulting clones those that include chromosomes with more than one centromere and/or fragments thereof. If desired, the heterologous nucleic acid includes sequences that target it to an amplifiable region, such as the pericentric region, of heterochromatin. Those of skill in the art, in working with conventional cytological techniques, have long described that herterochromatin can include the nucleolar organizer region comprising rDNA and have further described that rDNA found in the nucleolar organizing region can be identified within pericentric heterochromatin by the use of FISH, for example (see e.g., Hizume, M., *et al.*(1992) *Jpn. J. Genet.* 67: 389-396). Many different plant species are known to have rDNA sequences found within pericentric heterochromatin on acrocentric chromosomes (see e.g., Sato, S., *et al.* (1980) *Cytologia* 45: 87-96; Maluszynska J and Heslop-Harrison JS (1991) *Plant J.* 1: 159–166). In this Declaration, FISH images showing the pericentric localization of rDNA, in particular rDNA localized to the short arms of acrocentric chromosomes found in *Nicotiana* and *Brassica* plant species, is included.

8) In my capacity as researcher and Director of Research, I have personally supervised experiments related to the production of plant SATACs from two distinct plant species: *Nicotiana* and *Brassica*. *Nicotiana* is a species of plant from the core eudicots group, namely the Solanales within the euasterids I group of the asterids clase, which encompass nearly one-third of all angiosperm species. *Brassica* is a species of plant (a cruciferous plant) from the eurosids II group of the rosid clade, which encompass close to one-third of all angiosperm species. Numerous SATACs, and also precursors and

intermediates, such as dicentric chromosomes and sausage chromosomes, in the formation of SATACs have been generated in these plant species. Thus, plant SATACs have been successfully generated in distantly related plant species.

9) Using methods and materials described in the above-referenced application, and standard methods as described herein, myself and other scientists involved in these projects have generated multiple plant SATACs by introduction of DNA into plant cells as taught in the application. Analysis of the transfected plant cells, identified the presence of plant SATACs, and precursors and intermediates in the formation of plant SATACs including "sausage" chromosomes and dicentric chromosomes.

Hence, as described in the application, SATACs can be generated by producing cells that contain dicentric or multicentric chromosomes; and then culturing cells. When cultured, dicentric and multicentric chromosomes break to form a minichromosome and a formerly dicentric (or multicentric) chromosome, which after amplification events results in a SATAC. These methods described in the application have been employed to generate SATACs in plants as described in the application. As described in the application and based on the teachings of the specification, no knowledge of centromere DNA sequences is required to generate a SATAC in any species, including a plant SATAC.

Also, the results of this work demonstrate that a plant satellite artificial chromosome generated following the teachings in the above-captioned application possess the structural, physical and functional characteristics of a plant satellite artificial chromosome (*i.e.* a plant SATAC) described in the above-captioned application.

A description of the above-referenced methods and and the resulting production of several different SATACs in two different plant species is described in the following sections.

I. MATERIALS AND METHODS

A. Generation of Plant SATACs in *Nicotiana*

1. Construction of Heterologous DNA

a. Selectable Marker DNA

The nucleic acid sequence encoding a selectable marker was constructed by routine recombinant techniques by joining the Arabidopsis polyubiqutin 10 (UBQ10) 5' and 3' flanking regions (Norris *et al.* (1993) *Plant Mol Biol.*, 21:895-906) to the phosphinothricin N-acetyltransferase (PAT) gene (Wohlleben W *et al.* (1988) *Gene*, 70:25-37), carried in a pBluescript backbone (Stratagene, La Jolla, CA). This resulted in a selectable marker

construct conferring constitutive resistance to the herbicidal compound L-phosphinothricin (L-PPT) referred to as pDAB 2416. The nucleotide sequence of this vector is shown in Exhibit A.

b. Targeting DNA to the pericentric region

A targeting DNA molecule was constructed to target the integration of the selectable marker to the pericentric region, in particular the pericentric rDNA, of an acrocentric chromosome. The coding region of the 26S rDNA was chosen as a targeting sequence because it is highly conserved among species and it encodes a structural RNA molecule highly conserved among eukaryotic organisms. There are multiple sources of rDNA coding sequences available, dating back as early as 1985 (see *e.g.*, Takaiwa *et al.* (1985) Gene, 37:255-9.)

The coding region of a plant 26S rDNA (approximately 1.7 Kb) was chosen and isolated from the Arabidopsis rDNA repeat using published sequence and restriction mapping information (Genbank Accession no. X52320, which was deposited in the early 1990s; see also Pruit and Meyerowitz (1991) J Mol Biol., 187:169-83; Genbank Accession no. X15550; Gruender et al. (1991) J Mol Biol. 221:1209-1222). The targeting DNA was cloned into vector pBluescript (Stratagene, La Jolla, CA).

2. Introduction of DNAs into plant cells, selection and identification of amplified DNA

DNA encoding the selectable marker and targeting DNA were introduced into *Nicotiana* protoplasts using standard methods. Using standard methods, the DNA fragment encoding the selectable marker, comprised in the pDAB 2416 construct as shown in Exhibit A, was gel purified and introduced along with targeting DNA comprising the coding sequence of the 26S rDNA. In these experiments, both the selectable marker and targeting DNA were introduced into plant cells free of vector backbone, and were introduced into plant cells using PEG mediated transfection.

To illustrate the general method, tobacco cells were used. Briefly, tobacco protoplasts were isolated from established sterile tobacco plant cultures by immersion of sterile tissue in enzyme solution containing 1.2% Cellulase 'Onozuka' R-10 and 0.4% Macerozyme R-10. The protoplasts were purified by pouring through a 100 um nylon mesh sieve, overlayed with washing solution and centrifuged at 80xg for 10 min. Protoplasts were then resuspended at a density of $1x10^6$ protoplasts/ml and stored at 4°C for 1 to 2 hours prior to DNA uptake. Plasmid DNAs from the vector and targeting DNA were sterilized with

chloroform and 70% ethanol before use for transfection. A protoplast suspension was mixed with vector and targeting DNA at a ratio of 1:10 followed immediately by slowly adding PEG solution. Typically 30 μg of DNA mixture (vector and targetting DNA at a 1:10 ratio) were used per 1 X 10^6 protoplasts. The mixture was incubated at 22°C for 10-15 minutes with gentle shaking. The protoplasts were resuspended and cultured at 22°C in the dark. When microcalli developed, the protoplasts were embedded in 0.6% agarose.

Selection on protoplast cultures was carried out by adding L-PPT to the medium at a final concentration of 10 mg/l, 14 to 21 days after transfection. Following selection, plant cells were analyzed at the molecular level by southern blot for the presence of the selectable marker. Plant cells also were analyzed at the cytological level for co-localization of the selectable marker to pericentric DNA, amplification of pericentric DNA and for the presence of SATACs. For example, two-color fluorescent in situ hybridization (FISH) was performed using two probes. The first probe recognized the selectable marker sequence and was visualized with a fluorescein isothiocyanate (FITC) tag (yellow-green fluorescence). The second probe was tagged with rhodamine (red fluorescence) and recognized pericentric DNA (18S or 26S rDNA) sequences endogenous to *Nicotiana* cells.

To obtain spreads of metaphase chromosomes, cells were subjected to either a single blocking protocol (colchicine treatment) or double blocking protocol (for example, treating plant cells with 5 mg/l aphidocolin for 24 hours and then 1.54 mg/ml Propyzamide for 4 hours.) Blocked cells were recovered and chromosome spreads prepared and subjected to two-color FISH. Red and yellow-green fluorescence was monitored to identify amplification. The chromosome spreads were analyzed for the presence of structural features of chromosomes.

B. Generation of Plant SATACs in *Brassica napus*

The methods for generating plant SATACs are not unique to any singular selectable marker or plant species. Using methods as set forth in the above-referenced application, and as set forth above for the generation of SATACs in *Nicotiana*, heterologous DNA also was introduced into *Brassica napus* to produce plant SATACs, following selection and amplification of pericentric DNA.

The heterologous DNA included a construct containing a CaMv 35S promoter fused to a phosphinothricin acetyl transferase gene (*bar)* as a selectable marker (White *et al.* (1989) *Nucleic Acids Res.*, 18:1062), with an *att B* recombination site between the 35S promoter and *bar* selection gene, and was contained in a pBluescript backbone (Stratagene, La Jolla, CA),

referred to as pABI 012. The nucleotide sequence of this selectable marker is shown in Exhibit B. The DNA encoding the *bar* selectable marker was introduced along with 26S targeting DNA, as described above for generation of plant SATACs in *Nicotiana.* Typically 1-10 X 10^6 mesophyl protoplasts were isolated and used for DNA uptake. *Brassica napus* protoplasts were isolated from mesophyll material derived from *in vitro* cultured shoots essentially as described by Vamling K. and Glimelius K. (see e.g., Legumes and Oilseed Crops I in Biotechnology in Agriculture and Forestry 10, Springer Verlag (1990)). As done with *Nicotiana* species, plasmid DNAs from the vector and targeting DNA were sterilized with chloroform and 70% ethanol before use for transfection. A protoplast suspension was mixed with vector and targeting DNA at a ratio of 1:10 followed immediately by slowly adding PEG solution. Typically, 30 μg of DNA mixture (vector and targeting DNA at a 1:10 ratio) were used per 1 X 10^6 protoplasts.

Selection was on L-PPT and the plants were regenerated used standard protocols known in the art, *e.g.* those described in: Vamling K. and Glimelius K. (see *e.g.*, Legumes and Oilseed Crops I in Biotechnology in Agriculture and Forestry 10, Springer Verlag (1990); Glimelius K. *et al.* (1986) *Plant Sci.* 45, 133 – 144; Barsby *et al.* (1986) *Plant Cell Reports 5*, 101). Typically, up to 50% of L-PPT resistant calli regenerated to shoots and whole plants. The resultant spreads of chromosomes were analyzed by two-color FISH for the presence of chromosome structures as described above for *Nicotiana*, and in the above-referenced application.

II. Results

A. *Nicotiana*

The results show that by using the methods as described in the specification in plant cells, chromosomes were generated having the characteristic structural features (e.g., arms and a centromeric region) of chromosomes based on the description of satellite artificial chromosome generation in the above-captioned application and parent application U.S. Application Serial No. 08/629,822. These results provide evidence that homologous recombination between the exogenous DNAs and rDNA of the *Nicotiana* chromosomes had occurred, and that large scale amplification of pericentric DNA resulted. For example, a comparison of the yellow-green signal (to detect the selectable marker) and red staining (to detect 18S rDNA) of the same chromosome spread revealed overlap of the signals. Areas where significant levels of both yellow-green and red signals were observed demonstrate

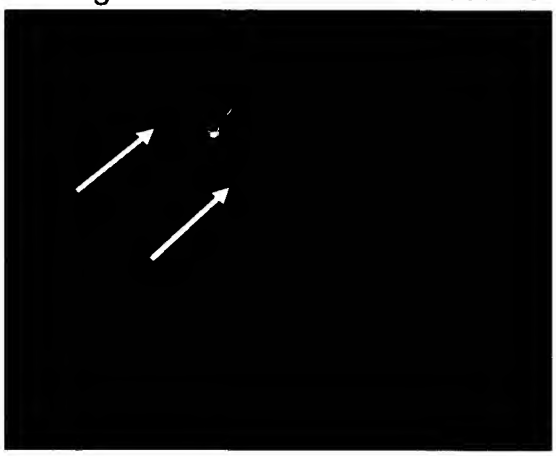
large-scale amplification of the pericentric DNA. This includes production of "sausage" chromosomes and amplification of heterochromatin satellite DNA, such as pericentric rDNA.

Over 20 independent SATACs or precursors and intermediates in the formation of SATACs (i.e. dicentric chromosomes or sausage chromosomes) have been generated in *Nicotiana*. Some exemplary chromosome structures are described below.

1. Dicentric chromosome

Figure 1 depicts a cell line whereby insertion of vector DNA into the pericentric heterochromatic DNA (rDNA) resulted in formation of a dicentric chromosome. As there are no centromere specific probes available for *Nicotiana*, a cytological observation by FISH analysis of centromere constriction was used to identify dicentric chromosomes. The chromosome in Figure 1 was stained with DAPI (blue) and co-stained for the presence of the selectable marker (FITC: yellow-green) and pericentric heterochromatic DNA (Rhodamine:red). The results show that the chromosome containing the amplified pericentric DNA contains two regions that show typical condensation and constriction indicative of a centromere region.

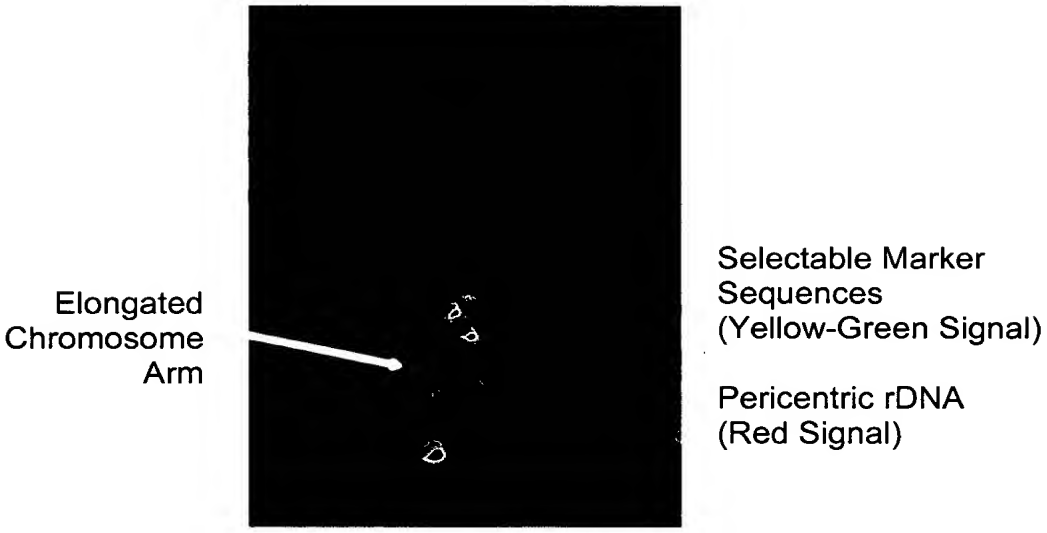
Figure 1: Dicentric Chromosome



2. Sausage Chromosome

Figure 2 depicts cytological analysis demonstrating the presence of a "sausage" chromosome generated in *Nicotiana* according to the method described herein, and in the above-referenced application. The chromosome in Figure 2 was stained with DAPI (blue) and co-stained for the presence of the selectable marker (FITC: yellow-green) and pericentric heterochromatic DNA (Rhodamine:red). The elongated chromosome arm depicted in Figure 2 demonstrates a periodicity of signal consistent with the "sausage" chromosome.

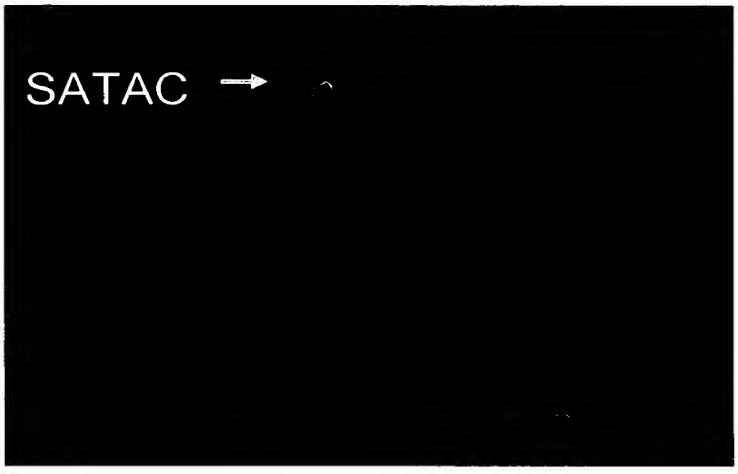
Figure 2: Sausage Chromosome



3. SATAC

Figure 3 depicts the results of cytological analysis evidencing the presence of a typical SATAC generated in *Nicotiana* according to the method described herein. FISH analysis demonstrates the presence of a chromosome containing multiple copies of the selectable marker (FITC: yellow-green) and a substantial amount of amplified pericentric heterochromatic DNA (Rhodamine: red) on a distinct chromosome structure. This evidences the generation of a SATAC.

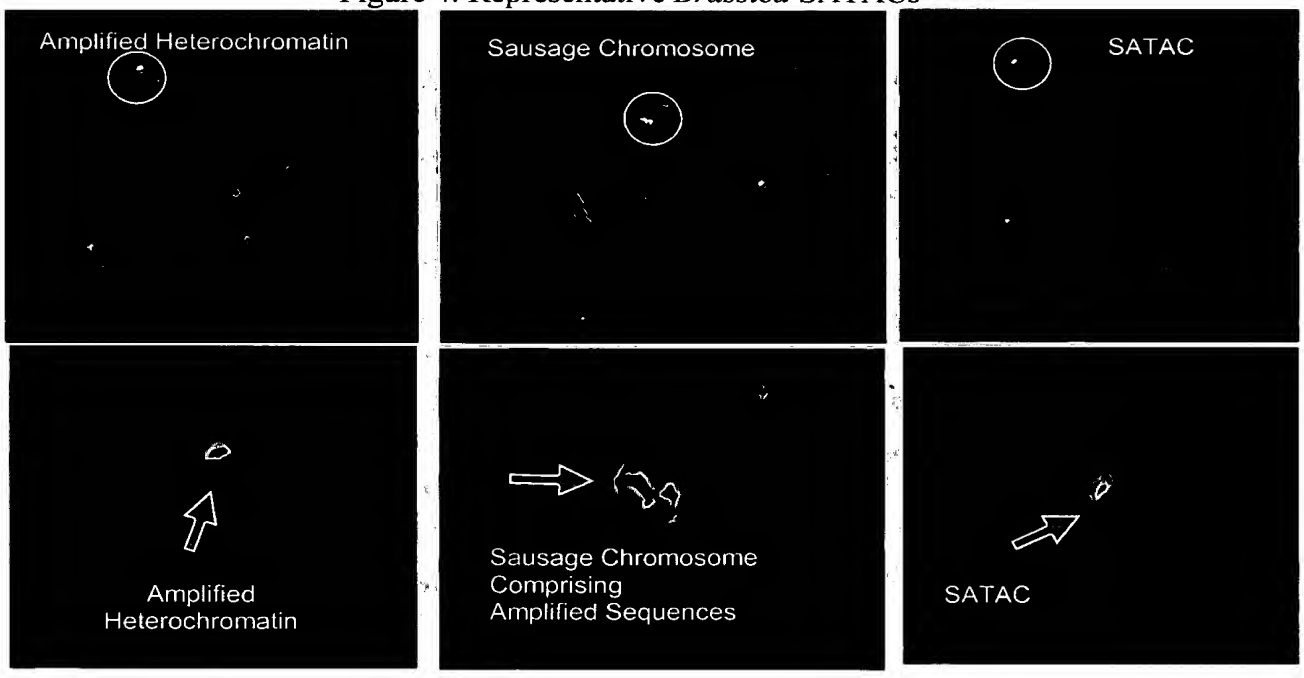
Figure 3: Typical SATAC



B. *Brassica napus*

Plant SATACs also were generated in *Brassica napus*. Figure 4 shows the results of FISH analysis of a root tip spread from a *Brassica* plant following introduction of heterologous DNA under selective conditions. The results in Figure 4 show that amplified selectable marker sequences are within amplified pericentric DNA evidencing homologous recombination between the exogenous DNAs and rDNA of the *Brassica* chromosomes. Southern blot analysis indicated that the selectable marker is represented at greater than 20 copies within this structure. Multiple structures of chromosomes having identifying characteristics as described in the above-referenced application for the generation of SATACs, were observed in *Brassica,* following selection and regeneration to whole plants. More than 10 SATACs and precursors and intermediates in the formation of SATACs have been generated and identified in *Brassica.* Some exemplary chromosome structures are depicted in Figure 4. As above, the chromosome spreads were stained with DAPI (blue) and co-stained for the presence of the selectable marker (FITC: yellow-green) and pericentric heterochromatic DNA (Rhodamine: red).

Figure 4: Representative *Brassica* SATACs



III. Conclusion

The results of the experiments provided herein demonstrate that by following the teachings of the specification and employing standard methods as described herein, plant satellite artificial chromosomes can be generated and selected within plants. The teachings of the specification provide a means to target a selectable marker to pericentric heterochromatin and to recover chromosomes that have amplified pericentric DNA, including dicentric or multicentric SATACs. The nature of the DNA sequences that can be employed are not limited to any particular DNA molecule. Further, the results herein depict that the method is broadly applicable to all plant species, including the distinct species of plants of *Nicotiana* and *Brassica*. Accordingly, the experiments described herein demonstrate the generation of satellite artificial chromosomes in plants using methods as taught in the application, resulting in chromosomes having the same identifying characteristics and structural features as taught in the application.

Applicant : Gyula Hadlaczky et al. Attorney's Docket No.: 17084-004006/402E
Serial No. : 09/724,726
Declaration

I further declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeapardize the validity of the application or any patent resulting therefrom.

May 28 2007
Date

Steven P. Fabijanski

Exhibit A: Annotated Nucleotide Sequence of pDAB 2416 Selectable Marker Vector

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   1  GTCGACCTGC AGGTCAACGG ATCAGGATAT TCTTGTTTAA GATGTTGAAC TCTATGGAGG TTTGTATGAA CTGATGATCT AGGACCGGAT AAGTTCCCTT CTTCATAGCG
      >>.....................................................AtUbi10 Pro v2.....................................................>

 111  AACTTATTCA AAGAATGTTT TGTGTATCAT TCTTGTTACA TTGTTATTAA TGAAAAAATA TTATTGGTCA TTGGACTGAA CACGAGTGTT AAATATGGAC CAGGCCCCAA
      >.....................................................AtUbi10 Pro v2.....................................................>

 221  ATAAGATCCA TTGATATATG AATTAAATAA CAAGAATAAA TCGAGTCACC AAACCACTTG CCTTTTTTAA CGAGACTTGT TCACCAACTT GATACAAAAG TCATTATCCT
      >.....................................................AtUbi10 Pro v2.....................................................>

 331  ATGCAAATCA ATAATCATAC AAAAATATCC AATAACACTA AAAAATTAAA AGAAATGGAT AATTTCACAA TATGTTATAC GATAAAGAAG TTACTTTTCC AAGAAATTCA
      >.....................................................AtUbi10 Pro v2.....................................................>

 441  CTGATTTTAT AAGCCCACTT GCATTAGATA AATGGCAAAA AAAAACAAAA AGGAAAAGAA ATAAAGCACG AAGAATTCTA GAAAATACGA AATACGCTTC AATGCAGTGG
      >.....................................................AtUbi10 Pro v2.....................................................>

 551  GACCCACGGT TCAATTATTG CCAATTTTCA GCTCCACCGT ATATTTAAAA AATAAAACGA TAATGCTAAA AAAATATAAA TCGTAACGAT CGTTAAATCT CAACGGCTGG
      >.....................................................AtUbi10 Pro v2.....................................................>

 661  ATCTTATGAC GACCGTTAGA AATTGTGGTT GTCGACGAGT CAGTAATAAA CGGCGTCAAA GTGGTTGCAG CCGGCACACA CGAGTCGTGT TTATCAACTC AAAGCACAAA
      >.....................................................AtUbi10 Pro v2.....................................................>

 771  TACTTTTCCT CAACCTAAAA ATAAGGCAAT TAGCCAAAAA CAACTTTGCG TGTAAACAAC GCTCAATACA CGTGTCATTT TATTATTAGC TATTGCTTCA CCGCCTTAGC
      >.....................................................AtUbi10 Pro v2.....................................................>

 881  TTTCTCGTGA CCTAGTCGTC CTCGTCTTTT CTTCTTCTTC TTCTATAAAA CAATACCCAA AGCTTCTTCT TCACAATTCA GATTTCAATT TCTCAAAATC TTAAAAACTT
      >.....................................................AtUbi10 Pro v2.....................................................>

 991  TCTCTCAATT CTCTCTACCG TGATCAAGGT AAATTTCTGT GTTCCTTATT CTCTCAAAAT CTTCGATTTT GTTTTCGTTC GATCCCAATT TCGTATATGT TCTTTGGTTT
      >.....................................................AtUbi10 Pro v2.....................................................>

1101  AGATTCTGTT AATCTTAGAT CGAAGACGAT TTTCTGGGTT TGATCGTTAG ATATCATCTT AATTCTCGAT TAGGGTTTCA TAAATATCAT CCGATTTGTT CAAATAATTT
      >.....................................................AtUbi10 Pro v2.....................................................>

1211  GAGTTTTGTC GAATAATTAC TCTTCGATTT GTGATTTCTA TCTAGATCTG GTGTTAGTTT CTAGTTTGTG CGATCGAATT TGTCGATTAA TCTGAGTTTT TCTGATTAAC
      >.....................................................AtUbi10 Pro v2.....................................................>

1321  AGGTAAGGAT CCAACCATGG CTTCTCCGGA GAGGAGACCA GTTGAGATTA GGCCAGCTAC AGCAGCTGAT ATGGCCGCGG TTTGTGATAT CGTTAACCAT TACATTGAGA
      >> AtUbi10 Pro v2
                     >>.....................................................PAT v3.....................................................>

1431  CGTCTACAGT GAACTTTAGG ACAGAGCCAC AAACACCACA AGAGTGGATT GATGATCTAG AGAGGTTGCA AGATAGATAC CCTTGGTTGG TTGCTGAGGT TGAGGGTGTT
      >.....................................................PAT v3.....................................................>

1541  GTGGCTGGTA TTGCTTACGC TGGGCCCTGG AAGGCTAGGA ACGCTTACGA TTGGACAGTT GAGAGTACTG TTTACGTGTC ACATAGGCAT CAAAGGTTGG GCCTAGGATC
      >.....................................................PAT v3.....................................................>
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1651  CACATTGTAC ACACATTTGC TTAAGTCTAT GGAGGCGCAA GGTTTTAAGT CTGTGGTTGC TGTTATAGGC CTTCCAAACG ATCCATCTGT TAGGTTGCAT GAGGCTTTGG
      >.....................................................PAT v3.....................................................>

1761  GATACACAGC CCGGGGTACA TTGCGCGCAG CTGGATACAA GCATGGTGGA TGGCATGATG TTGGTTTTTG GCAAAGGGAT TTTGAGTTGC CAGCTCCTCC AAGGCCAGTT
      >.....................................................PAT v3.....................................................>

1871  AGGCCAGTTA CCCAGATCTG AGGTACCCTG AGCTCGGATC CACTAGTAAC GGCCGCCAGT GTGCTGGAAT TCGCCCTTGA CTAGATAGGC GCCCAGATCG GCGGCAATAG
      >.......PAT v3.......>>
                                          >>.............................AtuORF1 3'UTR v3.............................>

1981  CTTCTTAGCG CCATCCCGGG TTGATCCTAT CTGTGTTGAA ATAGTTGCGG TGGGCAAGGC TCTCTTTCAG AAAGACAGGC GGCCAAAGGA ACCCAAGGTG AGGTGGGCTA
      >.....................................................AtuORF1 3'UTR v3.....................................................>

2091  TGGCTCTCAG TTCCTTGTGG AAGCGCTTGG TCTAAGGTGC AGAGGTGTTA GCGGGATGAA GCAAAAGTGT CCGATTGTAA CAAGATATGT TGATCCTACG TAAGGATATT
      >.....................................................AtuORF1 3'UTR v3.....................................................>

2201  AAAGTATGTA TTCATCACTA ATATAATCAG TGTATTCCAA TATGTACTAC GATTTCCAAT GTCTTTATTG TCGCCGTATG TAATCGGCGT CACAAAATAA TCCCCGGTGA
      >.....................................................AtuORF1 3'UTR v3.....................................................>

2311  CTTTCTTTTA ATCCAGGATG AAATAATATG TTATTATAAT TTTTGCGATT TGGTCCGTTA TAGGAATTGA AGTGTGCTTG CGGTCGCCAC CACTCCCATT TCATAATTTT
      >.....................................................AtuORF1 3'UTR v3.....................................................>

2421  ACATGTATTT GAAAAATAAA AATTTATGGT ATTCAATTTA AACACGTATA CTTGTAAAGA ATGATATCTT GAAAGAAATA TAGTTTAAAT ATTTATTGAT AAAATAACAA
      >.....................................................AtuORF1 3'UTR v3.....................................................>

2531  GTCAGGTATT ATAGTCCAAG CAAAAACATA AATTTATTGA TGCAAGTTTA AATTCAGAAA TATTTCAATA ACTGATTATA TCAGCTGGTA CATTGCCGTA GATGAAAGAC
      >.....................................................AtuORF1 3'UTR v3.....................................................>

2641  TGAGTGCGAT ATTATGGTGT AATACATAGC GGCCG
      >..........AtuORF1 3'UTR v3.........>>
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Exhibit B: Annotated Nucleotide Sequence of pABI 012 Selectable Marker Vector

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   1  CGACACTCTC GTCTACTCCA AGAATATCAA AGATACAGTC TCAGAAGACC AAAGGGCTAT TGAGACTTTT CAACAAAGGG TAATATCGGG AAACCTCCTC GGATTCCATT
      >>.....................................................CaMV-35S.....................................................>

 111  GCCCAGCTAT CTGTCACTTC ATCAAAAGGA CAGTAGAAAA GGAAGGTGGC ACCTACAAAT GCCATCATTG CGATAAAGGA AAGGCTATCG TTCAAGATGC CTCTGCCGAC
      >.....................................................CaMV-35S.....................................................>

 221  AGTGGTCCCA AAGATGGACC CCCACCCACG AGGAGCATCG TGGAAAAAGA AGACGTTCCA ACCACGTCTT CAAAGCAAGT GGATTGATGT GATAACATGG TGGAGCACGA
      >.....................................................CaMV-35S.....................................................>

 331  CACTCTCGTC TACTCCAAGA ATATCAAAGA TACAGTCTCA GAAGACCAAA GGGCTATTGA GACTTTTCAA CAAAGGGTAA TATCGGGAAA CCTCCTCGGA TTCCATTGCC
      >.....................................................CaMV-35S.....................................................>

 441  CAGCTATCTG TCACTTCATC AAAAGGACAG TAGAAAAGGA AGGTGGCACC TACAAATGCC ATCATTGCGA TAAAGGAAAG GCTATCGTTC AAGATGCCTC TGCCGACAGT
      >.....................................................CaMV-35S.....................................................>

 551  GGTCCCAAAG ATGGACCCCC ACCCACGAGG AGCATCGTGG AAAAAGAAGA CGTTCCAACC ACGTCTTCAA AGCAAGTGGA TTGATGTGAT ATCTCCACTG ACGTAAGGGA
      >.....................................................CaMV-35S.....................................................>

 661  TGACGCACAA TCCCACTATC CTTCGCAAGA CCTTCCTCTA TATAAGGAAG TTCATTTCAT TTGGAGAGGA CACGCTGAAA TCACCAGTCT CTCTCTACAA ATCTATCTCT
      >.....................................................CaMV-35S.....................................................>>
                                                                                                                    Att B <

 771  GTCGAGTGAA GCCTGCTTTT TTATACTAAC TTGAGCGAAC TCGAGTCTAC CATGAGCCCA GAACGACGCC CGGCCGACAT CCGCCGTGCC ACCGAGGCGG ACATGCCGGC
      <.....................Att B...................<<
                                                      >>.............................Bar.............................>

 881  GGTCTGCACC ATCGTCAACC ACTACATCGA GACAAGCACG GTCAACTTCC GTACCGAGCC GCAGGAACCG CAGGAGTGGA CGGACGACCT CGTCCGTCTG CGGGAGCGCT
      >.....................................................Bar.....................................................>

 991  ATCCCTGGCT CGTCGCCGAG GTGGACGGCG AGGTCGCCGG CATCGCCTAC GCGGGCCCCT GGAAGGCACG CAACGCCTAC GACTGGACGG CCGAGTCGAC CGTGTACGTC
      >.....................................................Bar.....................................................>

1101  TCCCCCCGCC ACCAGCGGAC GGGACTGGGC TCCACGCTCT ACACCCACCT GCTGAAGTCC CTGGAGGCAC AGGGCTTCAA GAGCGTGGTC GCTGTCATCG GGCTGCCCAA
      >.....................................................Bar.....................................................>

1211  CGACCCGAGC GTGCGCATGC ACGAGGCGCT CGGATATGCC CCCCGCGGCA TGCTGCGGGC GGCCGGCTTC AAGCACGGGA ACTGGCATGA CGTGGGTTTC TGGCAGCTGG
      >.....................................................Bar.....................................................>

1321  ACTTCAGCCT GCCGGTACCG CCCCGTCCGG TCCTGCCCGT CACCGAGATT TGACTCGAGT TTCTCCATAA TAATGTGTGA GTAGTTCCCA GATAAGGGAA TTAGGGTTCC
      >.........................Bar.........................>>
                                                              >>.....................CaMV polyA.....................>

1431  TATAGGGTTT CGCTCATGTG TTGAGCATAT AAGAAACCCT TAGTATGTAT TTGTATTTGT AAAATACTTC TATCAATAAA ATTTCTAATT CCTAAAACCA AAATCCAGTA
      >.....................................................CaMV polyA.....................................................>

1541  CTAAAATCCA GATCCCCCGA ATTAATTCGG CGTTAATTCA G
      >................CaMV polyA................>>
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